THE REACTION OF ANIMAL CELLS TO CHEMICAL STIMULI.

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AS the field of zoology broadens, our increased knowledge of the structure and behavior of animal forms drives us deeper and deeper into perplexing problems of life phenomena. The solution of many of these problems is looked for in the study of the simpler unicellular organisms. The fact that each one of these single-celled creatures is a living unit and performs all of the processes necessary to the maintenance of life makes them an attractive field of investigation.

It is the purpose of this paper to give a review of the work which has been done on the Protozoa in their relation to chemical stimuli and to give a few of the author's experiments on paramœcium. Two different classes of experiments have been carried on—first, the effect of temporary chemical stimulation on the individual animal; and second, the effect of both temporary and continued chemical stimulation on the race. The second class of experiments is manifestly more important.

The effect of chemical stimulation on the individual has been worked out by Jennings on various Protozoa. He has shown that the theory of tropisms, including chemotropism, is false when applied to the ciliates. An attempt was made by earlier investigators to make the reactions of the Protozoa conform to the tropism scheme. Tropism, in this case, chemotropism, is a direct movement or orientation of the animal towards, or away from, a diffusing chemical. In this movement the lines of diffusion bear a direct relation to the position and direction which an animal takes in moving under the influence of a chemical.

There is no doubt about the occurrence of chemotropism among plants, but no animal whose behavior has been carefully studied shows this simple reaction to chemicals. Jennings has demonstrated this fact. Among the protozoans some chemicals stimulate a motor reflex. This reflex action is a sudden backing, followed by a rapid turning of the animal toward a particular side, the one opposite the mouth. When the concentration is great enough, most chemicals start this motor reflex. In a majority of cases this action removes the animal from the source of stimulus, because the

anterior end comes in contact first. But, as Jennings has shown, if the animal is stimulated from behind, from the side or from any localized area the same reflex action takes place, even though it carries the animal into the midst of the chemical.

Some chemicals in weak solution do not stimulate the motor reflex. These are mostly those of an acid reaction. I have experimented with 0.0005 per cent. HCl and 0.00002 per cent. HCl. Several trials were made. The stronger solution did not cause the reflex, but it caused the animal to hesitate before entering. The weaker solution seemed to attract, from the fact that on contact the animal immediately swam into it. Two small drops were placed side by side, one containing the acid solution and the other paramœcium in water. Now, by the aid of a needle or a fine glass rod, the two drops are connected by a narrow neck. In its random course the little animal comes in contact with the diffusing chemical, but instead of backing off immediately it hesitates, perhaps turning toward the aboral side a few times and then entering the drop. This turning toward the aboral side is a normal movement.

After entering the drop the paramœcium darts about, showing signs of stimulation. If the connection between the two drops is small, so that the diffusion of the chemical is slow, the paramœcium, upon starting to reenter the drop of water, is repelled by having its motor reflex stimulated. The result is that the acid drop acts as a trap. If, on the other hand, the connection between the drops is large enough to permit a rapid diffusion of the chemical, instead of always being repelled by the drop of water, the animal often reenters.

The explanation is easily found. In the first instance the diffusion of the chemical is so slight that the two drops remain very different in chemical composition. Now, if an animal attempts to pass back into the drop of water it encounters such a sudden change that its reflex is stimulated, and the animal backs away. In the second instance, where the diffusion of the chemical is rapid, the drops blend into each other, so that the animal encounters no sudden change, and therefore is not repelled. Paramœcium gave a similar reaction to weak acetic acid.

What seems most peculiar in this reaction to acids is that there is no repulsion in entering the acid, but after having entered it the paramœcium is repelled by the water which it has just left. This shows conclusively that weak acid solutions have a positive attraction for paramœcia.

It has been found by other investigators that paramœcia collect in CO_2 which has been placed in the water. The CO_2 renders the

water weakly acid. It has been shown also by them that the metabolism in paramœcium, as in other animal cells, produces CO_2. Let us now place quite a anumber of paramœcia on a slide and watch their movements. In a short time they collect in groups. Is this due to a social instinct? Because paramœcia are attracted by any weak solution which gives an acid reaction, it seems obvious that the congregation of the paramœcia is due to a formation of CO_2 by the metabolism of their bodies.

If we try another class of chemicals, those of an alkaline reaction, we find that they repel.

The effect of immersion in different chemicals varies. In a one-per-cent. solution of NaCl, the organisms revolve about on their short axis and then dart from one part of the drop to the other. This restless activity is followed by short periods of rest.

A one-per-cent. solution of $MgCl_2$ does not produce so great a stimulation. Paramœcia, when immersed in this solution, dart back and forth for a short time, but soon quiet down and act normally. According to Jennings, paramœcia swim about in KI solution for six or seven minutes, giving the characteristic reflex action. This movement is followed by a continuous spinning which lasts for several minutes.

Curare produces a weakening effect after the stimulation, which lasts for about fifteen minutes. In this solution they alternate, running back and forth and spinning about, and finally become extremely quiet.

By these few examples we see how differently some chemicals affect the animal than do others. Some produce a prolonged backward movement, some produce a continued spinning, and others cause various modifications and combinations of these two movements.

Organic chemicals, such as sugars, gelatin, and urea, do not act on the motor reflex, but distend the infusorian and cause protrusions to arise on the exterior of the animal. I have experimented with sugar and gelatin. In connection with experiments with sugar a very interesting thing happened. I had a large ciliate which had been encysted for about nine months. After having tried several different methods to induce it to come out of the cyst, a solution of sugar was used. In about five hours the cyst had burst and the contents had begun to swell. The animal did not resume its active state, but immediately formed a new cyst. The osmotic pressure of the sugar undoubtedly caused the cyst to burst

and the protoplasm to swell. But the sugar solution not being suitable for the animal, it again fortified itself with a new cyst.

The behavior of the paramœcium is not radically different from that of the higher Metazoa, when subjected to chemical solution. The immediate effect is an increased activity. In many cases this is followed by a period of depression. Alcoholic stimulation in man is of the very same nature.

A study of the effect of chemicals on the individual leads one to wonder how chemical stimulation would affect the race. To determine this I have started a series of experiments. So far the chemicals used have been NaCl, $MgCl_2$, and curare. The results up to this time can be only tentative, because some of the experiments have not been carried on long enough.

On September 20 four lines of generation of paramœcia were started. Two lines were from a culture collected at Kansas City and two from a culture collected at Lawrence. In general, I used the methods which Calkins employed in his study of the life, history of paramœcium. For a culture medium I used a tea made by heating a small quantity of hay in ordinary tap water. This was allowed to stand for two or three days to allow bacteria to develop. Each paramœcium culture was kept in a drop of hay tea on an ordinary glass slide. These cultures were kept in a moist chamber to prevent evaporation. The division rate was watched from day to day. At every second or third generation a single individual was removed to a clean slide by the aid of a capillary pipette and new hay tea added. Perhaps the reason why paramœcium thrives so well in hay tea is because the chemicals and food are very much like those found in its natural habitat.

For convenience, we will call the four series of cultures A, B, C, and D. After these cultures had been running for twelve days and twenty-three generations had arisen, two single individuals of A and C were isolated on separate slides. Then a culture medium made up of three parts of hay tea to one part of one-per-cent. NaCl solution was placed on the slide for the animal to live in. Records and observations were made daily. Whenever the cultures were transferred the same amount of NaCl was added to the hay tea. The normal hay series, A and C, were used as check cultures. We will call the corresponding NaCl cultures Ax and Cx. At the end of the first twenty-four hours no division had occurred in either culture. In two days two divisions had occurred in Ax and one in Cx. In five days four generations had been produced in Ax and two in Cx. Cx divided no further, but died at the end of

nine days. Ax continued dividing slowly until the eleventh day, when it ceased dividing, and died on the twenty-second day. During this time the check cultures, A and C, had divided eighteen and six times, respectively.

The check culture C shows a low state of vitality, which accounts for the early death of Cx.

For comparison, we have in the NaCl culture Ax eight generations, as against eighteen generations of the hay check culture A; and in NaCl culture Cx we have two generations, against six of the hay check culture C.

This experiment shows conclusively that continued use of NaCl retards division and finally produces death. It may be safely inferred that the continued use of any chemical produces a decrease in the vitality.

I have also started a series of experiments to determine the effect of the stimulation of an individual on the race. The chemicals employed are one per cent. NaCl, one per cent. $MgCl_2$, and a weak solution of curare. The NaCl and curare cultures are now twenty-five days old and the $MgCl_2$ culture twenty-one days old. The NaCl and curare cultures were taken from the same generation of hay culture C, while the $MgCl_2$ culture is from D. Each culture has been stimulated twice by its respective chemical for periods varying from twenty to twenty-five minutes.

Up to the present time the NaCl and curare cultures show no decided variations from the check cultures. The $MgCl_2$ culture has shown a decided decrease in vitalty. The check culture has passed through seventeen generations while the chemically stimulated one has had only twelve generations. This shows that some chemicals decrease the vitality of the race, even when given in dilute solutions and only for a few minutes at a time.

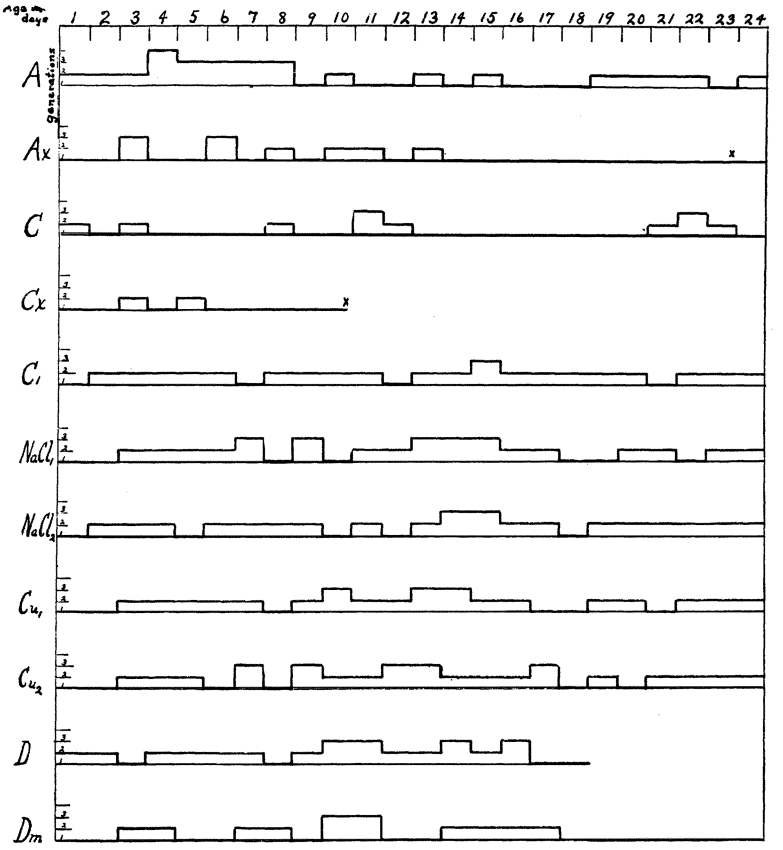
Calkins has succeeded in renewing the vitality of paramœcium by chemical stimulation when in periods of depression. He generally uses extracts from animal tissues, such as the brain, liver, and pancreas. Beef extract has been tested perhaps the most. In my own cultures, when there has been no division for six days, and the animals begin to show signs of dying, by stimulating with weak Liebig's beef extract the cultures have been restored to their normal vitality. Beef extract is not a food but a stimulant. Calkins shows that weak alcohol with continued use increases the division rate and tends to offset periods of depression. Also, that continued use of strychnine slightly increases the division rate and postpones death.

He also thinks that conjugation is a chemical stimulation which takes place by an interchange of nuclear material from individuals that have lived in different environments.

The chemical complex of the nucleus is perhaps determined by generations of varied environment and is influenced indirectly by immediate chemical change. Conjugation is probably in the nature of a chemical stimulus, but stimulation produced by crude chemical stimulation cannot produce the same result.

Loeb's well-known experiments on the unfertilized egg of the sea-urchin seems to give especial emphasis to the theory of chemical stimulation.

There is no doubt that chemical processes occupy an important place in life phenomena. Living organic matter is composed of highly complex organic compounds in a state of unstable equilibrium. Take away life and we place these complex compounds in stable equilibrium. The greater the vitality of an organism the more unstable is the equilibrium. When an organism begins to die it is approaching stable equilibrium. Slight variations in the chemical composition in the fluid supplied for the nourishment of a cell probably tends to maintain this unstable chemical equilibrium for a while; but in time this unstable equilibrium must be reenforced by a delicately complex stimulation from the nucleus of another individual.



Graphic representation of daily division rates of cultures, showing comparison between the chemically stimulated and the normal hay-fed series.

A, Ax, C, Cx, are the "NaCl continued stimulation series." Of these A and C are the check cultures, while Ax and Cx are the cultures stimulated continually with one per cent. NaCl. X marks the day on which death occurred.

C_1 is the check series for $NaCl_1$, $NaCl_2$, Cu_1, and Cu_2. $NaCl_1$ and $NaCl_2$ cultures were stimulated twice with one per cent. NaCl for periods of twenty-five minutes each. Cu_1 and Cu_2 cultures were stimulated twice with a weak solution of curare for periods of twenty minutes each.

D and Dm are the "$MgCl_2$ comparative series." D is the check culture, while Dm has been stimulated once with one per cent. $MgCl_2$ for twenty minutes.